CTRP5 (human) Competitive ELISA Kit

(Catalog #K4925-100; 100 assays; Store kit at 4°C)

I. Description:

CTRP5, a 25 kD secretory protein, is a member of the C1q and tumor necrosis factor superfamily whose structure resembles adiponectin. A RT-PCR study demonstrated CTRP5 expression in RPE, liver, lung, placenta, and brain and it has been proposed that a CTRP mutation (S163R) plays a critical role in affecting its higher order protein structure, potentially leading to a cause of abnormal adhesion between the RPE and Bruch membrane. Recent data indicates that CTRP is one of the genes highly induced by elimination of mitochondria and able to activate AMPK in a rat myotube cell line, L6. Stimulation of L6 with recombinant CTRP5, full length as well as globular domain, enhanced glucose uptake and fatty acid oxidation. These biochemical events did not seem to be mediated via AdipoR1 or AdipoR2, suggesting that a novel receptor(s) may exist for CTRP5 in this muscle cell line. Some CTRP members can physically interact with adiponectin, forming various multimeric structures. Therefore, measuring serum or plasma CTRP5 may provide important information on its involvement in novel metabolism. This assay is a competitive Enzyme Linked-Immunosorbent Assay (ELISA) for quantitative determination of human CTRP5 in biological fluids. A polyclonal antibody recognizing native human CTRP5 reacts with a series of predetermined recombinant human CTRP5 standard proteins or samples under competition in the human CTRP5-coated plate. Their relative reactivity is plotted with that of the standard proteins. This ELISA is specific for the measurement of natural and recombinant human CTRP5. It does not cross-react with mouse CTRP5, human CTRP2 (globular), human CTRP6, human CTRP7 (globular), human CTRP9 (globular), human CTRP10 (globular), mouse CTRP2 (globular), mouse CTRP9 (globular), human adiponectin, human adiponectin (globular), mouse adiponectin, mouse adiponectin (globular), rat adiponectin, rat adiponectin (globular), human RBP4, human Nampt, human vaspin, human GPX3, human ANGPTL3, human progranulin, human leptin. The assay range is 0.001 – 5 μ**g** CTRP5/ml and a detection limit of 1 ng/ml (based on adding two standard deviations to the mean value of the (50) zero standards).

II. Kit Contents:

Component	100 Assays	Part Number
Pre-coated Microtiter Plate	1 ea (12 x 8 well strips	K4912-100-1
Wash Buffer (10X)	50 ml	K4912-100-2
Diluent (5X)	50 ml	K4912-100-3
Detection Antibody	12 ml	K4912-100-4
Detector 100X (Hrp conjugated anti-IgG)	150 µl	K4912-100-5
Human CTRP5 Standard (lyophilized, 5 □g)	1 vial	K4912-100-6
Human CTRP5 QC Sample (lyophilized)	1 vial	K4912-100-7
TMB Substrate Solution	12 ml	K4912-100-8
Stop Solution	12 ml	K4912-100-10
Plate Sealers	3 each	K4912-100-11

III. Storage Conditions:

Reagents must be stored at 2 - 8°C when not in use. Bring reagents to room temperature before use. Do not expose reagents to temperatures greater than 25°C.

IV. Assay Procedure (Read the ENTIRE Protocol Before Proceeding)

1. Test Samples/Standards/QC Sample: (We recommend these be run in duplicate)

a) **Serum :** Use a serum separator tube. Let samples clot at room temperature for 30 min before centrifugation for 20 min at 1,000 x g. Assay freshly prepared serum or store serum in aliquots at ≤ -20°C for later use. Avoid repeated freeze/thaw cycles.

b) **Plasma :** Collect plasma using heparin, EDTA, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay freshly prepared plasma or store plasma sample in aliquot at ≤ -20°C for later use. Avoid repeated freeze/ thaw cycles. Note: **Serum, Plasma,** or **Cell Culture Supernatant** have to be diluted in Diluent 1X. Samples containing visible precipitates must be clarified before use.
Note: As a starting point, 1/2 dilution of serum or plasma is recommended. If samples fall the outside range of assay, a lower or higher dilution may be required.

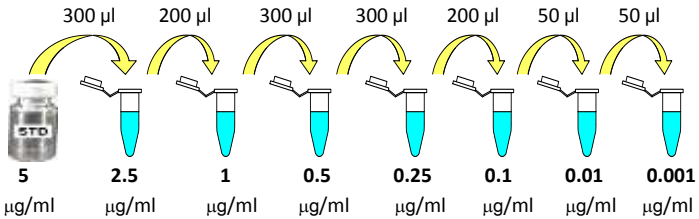
c) **QC Sample**: Reconstitute the human CTRP5 QC Sample with 1 ml of dH_2O. Mix the QC Sample to ensure complete reconstitution. Allow to sit for a minimum of 15 min. The QC Sample is ready to use-do not dilute it (refer to the C of A for current QC Sample concentration).

d) **Standards**: Reconstitute the human CTRP5 Standard with 1 ml of dH_2O to produce a stock solution (5 µg/ml). Mix the Stock solution to ensure complete reconstitution. Allow to sit for a minimum of 15 min. The reconstituted standard should be aliquoted and stored at -20°C.

e) Prepare 1X Diluent: Dilute 5X Diluent 1:4 with dH_2O.

f) Prepare Standard Curve using 2-fold serial dilutions with 1X Diluent:

To obtain	Add	Into
5 µg/ml	---	---
2.5 µg/ml	300 µl of CTRP5 (5 µg/ml)	300 µl of 1X Diluent
1 µg/ml	200 µl of CTRP5 (2.5 µg/ml)	300 µl of 1X Diluent
0.5 µg/ml	300 µl of CTRP5 (1 µg/ml)	300 µl of 1X Diluent
0.25 µg/ml	300 µl of CTRP5 (0.5 µg/ml)	300 µl of 1X Diluent
0.1 µg/ml	200 µl of CTRP5 (0.25 µg/ml)	300 µl of 1X Diluent
0.01 µg/ml	50 µl of CTRP5 (0.1 µg/ml)	450 µl of 1X Diluent
0.001 µg/ml	50 µl of CTRP5 (0.01 µg/ml)	450 µl of 1X Diluent



2. Reagent Preparation: (Prepare just the appropriate amounts for the assay)

a) **1X Wash Buffer:** Dilute 10X Wash Buffer 1: 9 with dH_2O to obtain 1X Wash Buffer.

b) **1X Diluent:** Dilute 5X Wash Buffer 1: 4 with dH_2O to obtain 1X Diluent.

c) **1X Detector**: Dilute 100X Detector 1: 99 with 1X Diluent to obtain 1X Detector.

Note: The diluted Detector must be used within 1 hr of preparation.

3. Assay Protocol:

a) Determine the number of 8-well strips needed for assay and insert them into the frame for current use. The extra strips should be resealed in the foil pouch and can be stored at 4°C for up to 1 month.

b) Add 50 µl of the Standards, Samples and QC Sample into the appropriate wells in duplicate.

c) Add 50 µl of the Detection Antibody to each well and tap gently on the side of the plate to mix.

d) Cover plate with plate sealer and incubate for 1 hr at 37°C.

e) Aspirate and wash x 3 with 300 µl of 1X Wash Buffer.

f) After last wash, tap inverted plate on a stack of paper towels. Complete removal of liquid is essential for good performance.

g) Add 100 µl of the 1X Detector to each well.

h) Cover plate with plate sealer and incubate for 1 hr at 37°C.

i) Remove plate from 37°C, aspirate and wash x 5 with 300 µl of 1X Wash Buffer.

j) After last wash, tap inverted plate on a stack of paper towels. Complete removal of liquid is essential for good performance.

k) Add 100 µl of the TMB Substrate Solution to each well.

l) Allow the color to develop at room temperature in the dark for 10 min.

m) Stop the reaction by adding 100 µl of Stop Solution to each well.

n) Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.
Caution: Stop Solution is a Corrosive Solution

o) Measure the OD at 450 nm in an ELISA plate reader within 30 min.

BioVision Incorporated
155 S. Milpitas Boulevard, Milpitas, CA 95035 USA

Tel: 408-493-1800 | Fax: 408-493-1801
www.biovision.com | tech@biovision.com

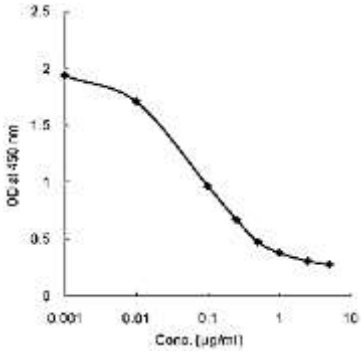
3. Calculations:

a) Average the duplicate readings for each Standard, QC Sample and Test Sample

b) Generate a Standard Curve by plotting the average absorbance on the vertical (Y) axis vs. the corresponding concentration (µg /ml) on the horizontal (X) axis. (See Typical Data below)

c) Calculate the Test Sample CTRP5 concentrations by interpolation of the Standard Curve regression curve as shown below in the form of a 4-parameter logistic equation.

d) If the Test Samples were diluted, multiply the interpolated values by the dilution factor to calculate the corrected human CTRP5 concentrations.



VI. Performance Characteristics:

1. Intra-assay Precision: (4) samples of known concentration were assayed in replicates (9) times to test precision within an assay.

Samples	Mean (µg/ml)	SD	CV (%)	n
1	0.351	0.02	5.87	9
2	0.293	0.03	10.00	9
3	0.250	0.01	4.05	9
4	0.165	0.01	6.06	9

2. Inter-assay Precision: (4) samples of known concentration were assayed in (3) separate assays to test precision between assays.

Samples	Mean (µg/ml)	SD	CV (%)	n
1	0.348	0.02	6.36	3
2	0.279	0.03	9.08	3
3	0.253	0.02	8.11	3
4	0.162	0.01	6.77	3

3. Linearity: Human serum samples containing CTRP5 were diluted several fold and the measured recoveries ranged from 79 % to 130 %.

Samples	Sample Dilution	Expected (µg/ml)	Observed (µg/ml)	% of Expected
1	1	0.260	0.260	100
	1:2	0.130	0.125	95
	1:4	0.065	0.071	108
2	1	0.174	0.174	100
	1:2	0.087	0.082	94
	1:4	0.044	0.056	127
3	1	0.321	0.321	100
	1:2	0.161	0.127	79
	1:4	0.080	0.071	88

Expected Values: CTRP5 levels range in plasma and serum from **0.05 to > 0.5 µg/ml** (Healthy donors)

Technical Hints and Limitations:

- It is recommended that all standards, QC sample and samples be run in duplicate.
- Do not combine leftover reagents with those reserved for additional wells.
- Reagents from the kit with a volume less than 100 µl should be centrifuged.
- Residual wash liquid should be drained from the wells after last wash by tapping the plate on absorbent paper.
- Crystals could appear in the 10X solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
- Once reagents have been added to the 8-well strips, DO NOT let the strips DRY at any time during the assay.
- Keep TMB Substrate Solution protected from light.
- The Stop Solution consists of phosphoric acid. Although diluted, the Stop Solution should be handled with gloves, eye protection and protective clothing.

Troubleshooting:

PROBLEM	POSSIBLE CAUSES	SOLUTIONS
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times inadequate	Incubation times should be followed as indicated in the manual.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use.
High background	Concentration of detector too high	Use recommended dilution factor.
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Poor standard curve	Wells not completely aspirated	Completely aspirate wells between steps.
	Reagents poorly mixed	Be sure that reagents are thoroughly mixed.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.

FOR RESEARCH USE ONLY! Not to be used on humans.